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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Assessment of axon health in spinal cord injury (SCI) is vital for proper diagnosis and treatment. Magnetic resonance imaging (MRI) is routinely performed in patients and provides valuable information about cord edema and hemorrhage. However, comprehensive prediction of axonal changes from in vivo MR imaging remains elusive. At the U. Penn site, we are applying two novel MRI methods to the problem of assessment of axonal loss, axonal diameter distribution, and myelin loss (q-space imaging (QSI) and ultra-short echo-time (UTE) MRI) first on animal specimens and then on human subjects.

During the reporting period we have further developed the method for myelin detection and quantification by direct observation of the myelin protons. However, given the hardware problems on the Bruker DFX-400 microimaging system (detailed in the report below) we have instead of focusing on measurements in the mouse spinal cord, worked toward myelin quantification on a clinical scanner, which will eventually enable measurements in humans. Specifically, we have been able to unambiguously detect and quantify the signal from extracted and regenerated bovine myelin using a novel zero-echo-time (ZTE) pulse sequence at 3 Tesla field strength.

15. SUBJECT TERMS
Axon Architecture, Spinal Cord Injury, Axon Loss, Myelin, Q-Space Imaging, UTE, MRI

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Table of Contents

	Page
Introduction……..…….…..	**1**
Body…….	**1-2**
Key Research Accomplishments…….…….	**2**
Reportable Outcomes……	**2**
Conclusion……	**2**

INTRODUCTION

Spinal cord injuries (SCI) produce direct mechanical disruption with subsequent severe degeneration of axons, and are the processes underlying the associated neurologic deficits observed in such injuries. Histological studies of fixed tissue in animal models of SCI have described axonal loss and demyelination occurring after trauma. Research at the University of Pennsylvania site brings novel magnetic resonance methodology to bear with the objective of obtaining quantitative information on axonal degeneration and myelin loss following spinal cord injury in a mouse model by pursuing the following Specific Aims per the work statement:

1. We will perform q-space MR imaging (QSI) and simulations of QSI to quantify axonal architecture in healthy and injured mouse spinal cords.
2. We will quantify myelin content with three quantitative MRI techniques in healthy and injured mouse spinal cords and compare the results with histology.

Specific Aim 1:

There was no progress on this specific aim for the following reasons:

1. After upgrade of the 400 MHz Bruker NMR/MRI the imaging pulse sequences developed previously were no longer compatible with the new software and the protocol therefore had to be redeveloped from scratch.
2. The custom-built gradient insert developed for the high-resolution q-space experiments in injured mouse spinal cords is irreparably damaged, a conclusion at which we arrived during the course of the reporting year. We therefore decided to port the protocol to the manufacturer-supplied gradients. While this will affect the achievable resolution for the measurement of axon size and integrity it should not impair our ability to distinguish injury from healthy spinal cords.
3. The postdoctoral fellow who had done the preliminary work has left the institution requiring recruitment of a successor, which took longer than expected.

We expect, with the aid of the unspent funds, which we requested to carry over into an additional program year, to complete the project by 10/2014.

Specific Aim 2:

We have continued to work toward methods for direct MRI detection and quantification of myelin, whose loss is a hallmark for spinal cord injury, following up on the research we reported in 2012 and that resulted in an article in the Proceedings of the National Academy of Science (Wilhelm et al, PNAS 2012). However, since we have not been able yet to put the pulse sequences in place for Q-space imaging and resolving the hardware issues with the home-built imaging gradients, we decided to defer measurements with the other targeted methods pending resolution of this issue. Instead, we have developed imaging pulse sequences and RF coils for myelin imaging at 3T on a human MRI scanner (Siemens TIM Trio), showing that we can detect and quantify the signal from the very short-lived MR signal of myelin (life-time $<<100\mu s$). Fig. 1 shows images of reconstituted myelin suspended in deuterium oxide (D_2O), along with an image of pure D_2O, conclusively demonstrating that the signal arises from the protons in myelin rather than residual HDO from incomplete deuteration. Further, the concentration of the myelin matched the concentration in white matter of the human spinal cord. Of note is that this coil is suited for imaging human-sized spinal cords ex vivo, as an important step toward myelin imaging of the spinal cord in vivo in patients with spinal cord injury.

We will during the period of the no-cost extension perform the same experiments in the injured murine spinal cords, after the pulse sequence has been modified to include water

suppression and also, after exchange of the native tissue water with D_2O (the success of which does not hinge on the effectiveness of the tissue water suppression).

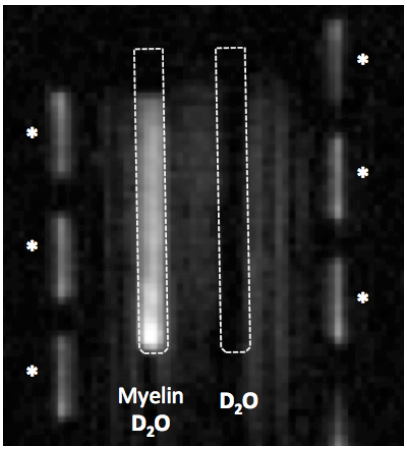

Fig. 1 3D ZTE proton image at 3T field strength of a sample of bovine myelin suspended in D_2O (70mg/550µl = 12.7%), co-imaged with a sample of neat D_2O in a 4.5 cm diameter solenoidal coil. The coil and surrounding structures are proton-free as described above (except for the adhesive on the copper tape, giving a faint signal outlining conductors (asterisks)). The image was reconstructed from 30,000 half-projections of 64 points each and average SNR was 48 at 1 mm^3 voxel size, obtained in 31 minutes. (An image of comparable SNR was obtained from 5,000 half-projections in 10 min scan time at the expense of some undersampling artifacts).

KEY RESEARCH ACCOMPLISHMENTS

- Implementation of hardware capabilities for direct myelin MRI at 3T field strength.
- Implementation of a 3D zero-echo-time (3D ZTE) imaging pulse sequence to operate on a commercial human MR imager.
- Demonstration of feasibility of detection of the short-lived protons in extracted and reconstituted myelin.

OUTCOMES

The new myelin imaging technique developed in this project has shown feasibility for quantitative assessment of myelin content in extracted and reconstituted myelin, with the technology readily transferable to the injured mouse spinal cord, a key goal of the project.

CONCLUSION

Even though the main objective to obtain data in the injured cord, and quantification of the hypothesized temporal changes following injury, has not been attained yet, we are confident to be able to complete both aims of the project during the requested no-cost extension period with the residual funds.